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ART 34 AMDT

## Claims

1. A plasmid vector for targeted transformation of filamentous  
5 fungi comprising
  - a) an origin of replication for a host organism not originating from the filamentous fungi to be transformed;
  - 10 b) a selection marker for a host organism not originating from the filamentous fungi;
  - 15 c) a promotor facilitating recombinant expression in fungi that is functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to a terminator which facilitates transcription termination in filamentous fungi;
- 20 wherein the overall size of the elements a), b) and c) does not exceed 4500 bp; and
- 25 d) a nucleic acid sequence which is homologous to nucleic acid sequences of the filamentous fungi to be transformed and makes homologous recombination in the filamentous fungi to be transformed possible.
2. A plasmid vector as claimed in claim 1, wherein the origin of replication a) originates from bacteria.
- 30 3. A plasmid vector as claimed in claims 1 to 2, wherein the selection marker b) imparts a resistance to antibiotics.
4. A plasmid vector according to claims 1 to 3, wherein the promotor of element c) is selected from the group consisting of  
35 the GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PHO5-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFa- and the NMT-promotor.
5. A plasmid vector according to claims 1 to 4, wherein the terminator of element c) is selected from the group consisting of the AOX1-, nos-, PGK-, TrpC- and the CYC1-terminator.  
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6. A plasmid vector according to claims 1 to 5, wherein the promotor of element c) is the GPD-1-promotor and the terminator of element c) is the nos-terminator.  
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7. A plasmid vector according to claims 1 to 6, wherein the nucleic acid sequence d) is functionally linked to a promotor facilitating recombinant expression in filamentous fungi.
- 5 8. A plasmid vector according to claims 1 to 7, wherein the nucleic acid sequence d) is functionally linked to a transcription terminator facilitating recombinant expression in filamentous fungi.
- 10 9. A selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, wherein said nucleic acid sequence comprises
- 15 i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or
- ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or
- 20 iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence that has at least an identity of 50% with the SEQ ID NO: or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 41% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 49% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in
- 25 SEQ ID NO:10 that has at least an identity of 6; or
- 30 iv. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp; or
- 35 v. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp comprising
- a) a nucleic acid sequence shown in SEQ ID NO:7 ; or
- 40 b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:8 by back translation; or

- c) a functional equivalent of a nucleic acid sequence set forth in a), which is encoded by amino acid sequence that has at least an identity of 85% with the SEQ ID NO:8.

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## 10. Use of a nucleic acid sequence comprising

- a) a nucleic acid sequence encoding a polyketide synthetase; or

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- b) parts of the nucleic acid sequence as defined in i. consisting of at least 300bp.

as marker for targeted transformation in filamentous fungi.

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## 11. Use of a nucleic acid sequence according to claim 10 said nucleic acid sequence comprising

- i. a nucleic acid sequence according to claim 9; or

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- ii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11; or

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- iii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

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- iv. a functional equivalent of the nucleic acid sequence set forth in i), which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of ; or

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- v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp; or

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- vi. parts of the nucleic acid sequence as defined in ii., iii or iv. consisting of at least 300bp comprising a nucleic acid sequence, which is encoded by an amino acid

sequence that has at least an identity of 68% with the SEQ ID NO:8.

12. A plasmid vector for targeted transformation of filamentous  
5 fungi additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, said nucleic acid sequence comprising
- i. a nucleic acid sequence according to claim 9; or  
10 ii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6.  
15 iii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;
- iv. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID  
20 NO:13 by back translation; or
- v. parts of the nucleic acid sequence as defined in ii.,  
25 iii. or iv. consisting of at least 300bp; or
- vi. parts of the nucleic acid sequence as defined in i., ii. or iii. or iv. consisting of at least 300bp, which are encoded by an amino acid sequence that has at least an identity of 68% with SEQ ID NO:8.  
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13. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, said nucleic acid sequence  
35 comprising
- i. a nucleic acid sequence according to claim 9; or
- ii. a functional equivalent of the nucleic acid sequence set  
40 forth in i), which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6; or
- iii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID  
45 NO: 11;

- iv. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or
- 5 v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp; or
- 10 vi. parts of the nucleic acid sequence as defined in i., ii. or iii. or iv. consisting of at least 300bp comprising a nucleic acid sequence, which is encoded by a functional equivalent of an amino acid sequence that has at least an identity of 68% with the SEQ ID NO:8.
- 15 14. An expression cassette comprising
- a) a promotor sequence in functional linkage with a nucleic acid sequence according to claim 9 in antisense orientation; and optionally
- 20 b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).
- 15 15. A plasmid vector for targeted transformation of filamentous fungi additionally comprising an expression cassette according to claim 14.
- 25 16. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising an expression cassette according to claim 14.
- 30 17. A method for transforming filamentous fungi, comprising the following steps
- 35 a) transferring a plasmid vector according to claim 12, 13, 15 or 16 into a filamentous fungi;
- b) selecting successfully transformed filamentous fungi by the absence of color.
- 40 18. An expression cassette comprising
- a) a promotor sequence in functional linkage with a nucleic acid sequence comprising
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- i. a nucleic acid sequence shown in SEQ ID NO:3, 4 or 5;  
or
- 5 ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or
- 10 iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 40% with the SEQ ID NO:6; or
- 15 iv. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;
- 20 v. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation;

and optionally

- 25 b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).

19. A method for transformation of filamentous fungi, comprising the following steps

- 30 a) providing a filamentous fungi, in which the polyketide synthetase gene is modified in such away that the polyketide synthetase cannot be functionally expressed;
- 35 b) transforming the filamentous fungi of step a) with an expression cassette according to claim 18 or a vector comprising the aforementioned expression cassette;
- 40 c) selecting successfully transformed filamentous fungi by the presence of color.

20. A method as claimed in claim 17 or 19, wherein the plasmid vector comprises at least an additional selection marker.

45 21. A method as claimed in claims 17, 19 or 20, wherein the selection is confirmed by PCR.

22. A method as claimed in claims 17, 19, 20 or 21; wherein the filamentous fungi are succesuflly transformed and identified in a high-throughput screening.

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